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Report Title

Biofilm formation by a metabolically versatile bacterium

ABSTRACT

Rhodopseudomonas palustris is a photosynthetic bacterium that has good potential to be developed as a biocatalyst for the production of hydrogen, a biofuel. The goal of this project is to conduct basic studies that will facilitate the development of a process wherein Rhodopseudomonas cells grown on surfaces as biofilms, produce hydrogen with energy provided from sunlight and electrons derived from agricultural waste. We characterized hydrogen production by Rhodopseudomonas, started to characterize biofilm formation by this species and initiated studies to identify the structure of a chemical signal that may activate expression of genes important for biofilm formation by Rhodopseudomonas.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Samanta, S. K and C. S. Harwood. 2005. Use of the Rhodopseudomonas palustris genome to identify a single amino acid that contributes to the activity of a coenzyme A ligase with chlorinated substrates. Mol. Microbiol. 55: 1151-1159.

Harrison, F.H. and C. S. Harwood. 2005. The pimFABCDE operon from Rhodopseudomonas palustris mediates dicarboxylic acid degradation and participates in anaerobic benzoate degradation. Microbiology 151:727-736.

Number of Papers published in peer-reviewed journals: 2.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

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International Workshop on Biorefinery, Kyoto, Japan, February, 2005

Biotechnology Workshop, Army Resrach office, Cashiers, North Carolina, May 2005

"Putting microbes to work", Annual ASM meeting, Atlanta, GA, June 2005

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Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED	
Faith Harrison	1.00	No
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED	
Sudip Samanta	0.50	No
FTE Equivalent:	**0.50**	
Total Number:	**1**	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Caroline Harwood	0.10	No
FTE Equivalent:	**0.10**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME	
Faith H. Harrison	No
Total Number:	**1**

Names of other research staff

NAME	PERCENT_SUPPORTED
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Biofilm formation by a metabolically versatile bacterium.

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Abstract

Rhodopseudomonas palustris is a photosynthetic bacterium that has good potential to be developed as a biocatalyst for the production of hydrogen, a biofuel. The goal of this project is to conduct basic studies that will facilitate the development of a process wherein *Rhodopseudomonas* cells grown on surfaces as biofilms, produce hydrogen with energy provided from sunlight and electrons derived from agricultural waste. We have characterized hydrogen production by *Rhodopseudomonas*, started to characterize biofilm formation by this species and initiated studies to identify the structure of a chemical signal that may activate expression of genes important for biofilm formation by *Rhodopseudomonas.*

Background and significance:

Rhodopseudomonas palustris is a photosynthetic bacterium that has good potential as a biocatalyst for hydrogen production by means of its nitrogenase enzymes. Hydrogen is produced concomitantly with ammonia as a product of nitrogen fixation. This process requires large amounts of ATP and electrons, which *Rhodopseudomonas* can derive from plant biomass and sunlight, respectively. It should thus be possible to configure bioreactors where *Rhodopseudomonas* cells illuminated by sunlight degrade agricultural waste and generate hydrogen as a product of nitrogen fixation. *Rhodopseudomonas* forms biofilms - defined as multicellular communities enclosed in a self-produced extracellular matrix - on surfaces when it is grown with the green plant-derived aromatic compound, 4-hydroxycinnamate. This is an important characteristic because biofilms provide a means of exposing large surface areas of cells to light, a prerequisite for hydrogen production. By analogy with other bacteria, *Rhodopseudomonas* likely controls the expression of genes involved in biofilm formation through a process called “quorum sensing”. *Rhodopseudomonas* encodes a quorum signal synthesis gene that is expressed when cells are grown to high cell densities in the presence of 4-hydroxycinnamate.

Statement of the problem studied:

To carry out studies of nitrogenase gene expression and hydrogen production by *Rhodopseudomonas*, 2) to characterize biofilm formation by this species and 3) to identify the chemical structure of the quorum-sensing signal that activates expression of genes that may be important for biofilm formation in *Rhodopseudomonas*. Progress on each of these topics is detailed as follows:

Summary of the most important results.

1: Studies of nitrogenase gene expression and hydrogen production by *Rhodopseudomonas.* We completed work aimed at analyzing nitrogenase gene

expression and hydrogen production by *Rhodopseudomonas palustris. R. palustris* is one of just a few prokaryotes so far described that has *vnf* and *anf* genes for alternative vanadium cofactor (V) and iron cofactor (Fe) nitrogenases, in addition to *nif* genes for a molybdenum cofactor (Mo) nitrogenase. Understanding the differential regulation of nitrogenase isozyme synthesis is an important step in the possible development of *R. palustris* as a biological catalyst for hydrogen production. We found that *R. palustris* strains expressing V nitrogenase or Fe nitrogenase catalyzed the production of two-fold and four-fold more hydrogen per nitrogen molecule reduced, than Mo nitrogenase strains. We used whole genome microarray expression analysis to show that the 32 genes in the *nif* gene cluster, but not the *anf* or *vnf* genes, were induced in wild-type and Mo nitrogenase-expressing strains grown under nitrogen-fixing conditions in Mo-containing medium. Strains that were unable to express a functional Mo nitrogenase due to mutations in Mo nitrogenase structural genes, synthesized functional V and Fe nitrogenases and expressed *vnf* and *anf* genes in nitrogen-fixing growth media that contained Mo and V at concentrations far in excess of those that repress alternative nitrogenase gene expression in other bacteria. Thus, not only does *R. palustris* have multiple enzymatic options for nitrogen fixation, but in contrast to reports from other nitrogen-fixing bacteria, expression of its alternative nitrogenases is not repressed by transition metals. Between 95 and 295 genes that are not directly associated with nitrogenase synthesis and assembly were induced under nitrogen-fixing conditions depending on which nitrogenase was being used by *R. palustris*. Genes for nitrogen acquisition were expressed at particularly high levels during alternative nitrogenase-dependant growth. This suggests that alternative nitrogenase-expressing cells are relatively starved for nitrogen and raises the possibility that fixed nitrogen availability may be the primary signal that controls the synthesis of the V and Fe nitrogenases.

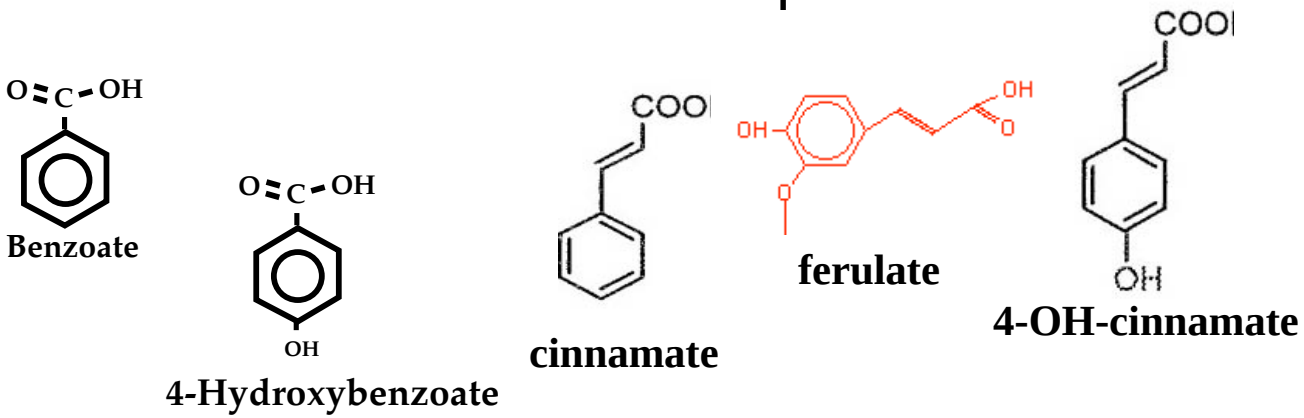
2: Biofilm formation by *R. palustris*. In work carried out this year we established that *R. palustris* forms robust biofilms on the sides of culture tubes only when it is grown in the presence of green-plant related aromatic compounds (Fig. 1). This is fortuitous since an ultimate goal of the project is to use decaying plant material as a source of electrons for hydrogen generation. We also established that cells in biofilms do in fact produce hydrogen on exposure to light when given an appropriate electron source (Fig. 2). A longer-term goal of the project is to grow biofilms on a glass surface in flow cell chambers and then to determine the spatial distribution of nitrogenase gene expression within mature structured biofilms. In initial experiments shown in Fig. 3, we established that cells do form biofilms in flow cell chambers.

3: Identification of the *R. palustris* quorum sensing signal. Some gram-negative bacteria secrete tiny amounts of small molecules called acyl-HSLs when they are growing. At a certain critical cell density sufficient acyl-HSL accumulates to turn on cell density dependant genes. This system, called quorum sensing, is generally used to activate genes that benefit cells when they are in groups, as in when they associated with plant or animal hosts. Quorum sensing controls or influences characteristics as diverse as biofilms, extracellular proteases, siderophores, antibiotic production, pectinases, exopolysaccharides, rhamnolipid synthesis, bioluminescence and root nodulation. The *R. palustris* genome includes a gene, *rpa0320*, that is a homologue of quorum signal synthesis (acyl-HSL synthesis) genes from other bacteria. One of the goals of this project was to determine the structure of the acyl-HSL quorum signal produced by *R. palustris*. The structure of the acyl-HSL produced by a given species of bacterium confers specificity so that a bacterium communicates only with its own kind. We have obtained evidence that *R. palustris* produces a new kind of acyl-HSL with a structure that is significantly different from that of all other acyl-HSLs that have been previously

characterized from other bacteria. In order to develop an assay so that we could follow the purification of the specific type of acyl-HSL produced by *R. palustris*, we constructed a reporter strain by inserting a *lacZ*::Km^R cassette in its acyl-HSL synthase gene. This acyl-HSL reporter strain synthesizes beta-galactosidase when extracts of wild-type *R. palustris* cultures are added exogenously, but not when extracts from an *rpa0320* mutant are added. Using this new reporter strain to detect putative acyl-HSL signals, we have separated *R. palustris* extracts via HPLC and identified two major peaks of activity. The active fractions are currently being analyzed by mass spectrometry. The results should allow us to assign a structure to the *R. palustris* quorum-sensing signal(s).

Fig. 1.

Biofilm formation is specific to plant derived aromatic compounds.



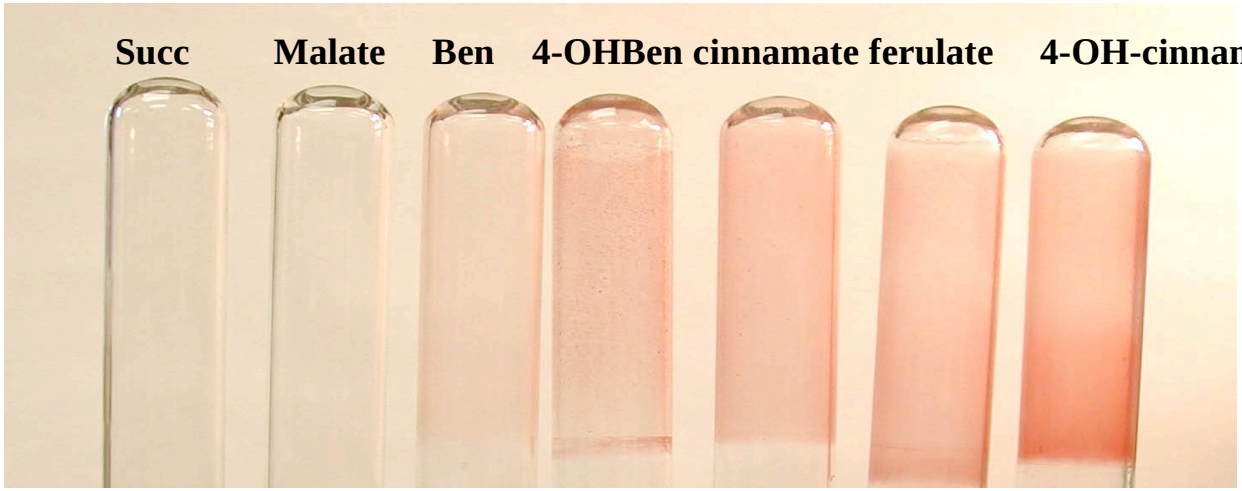
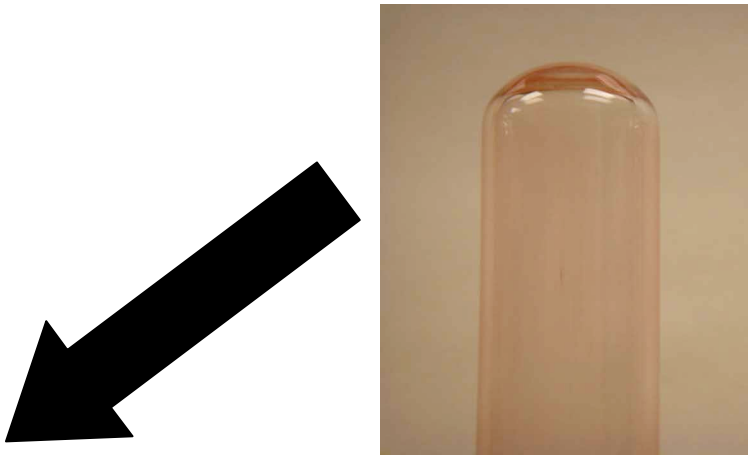
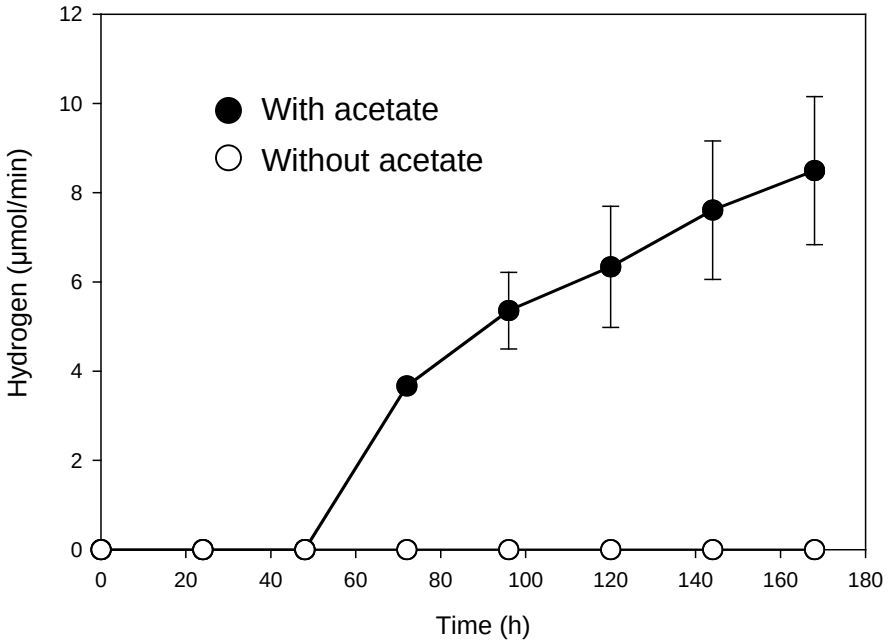


Fig. 2



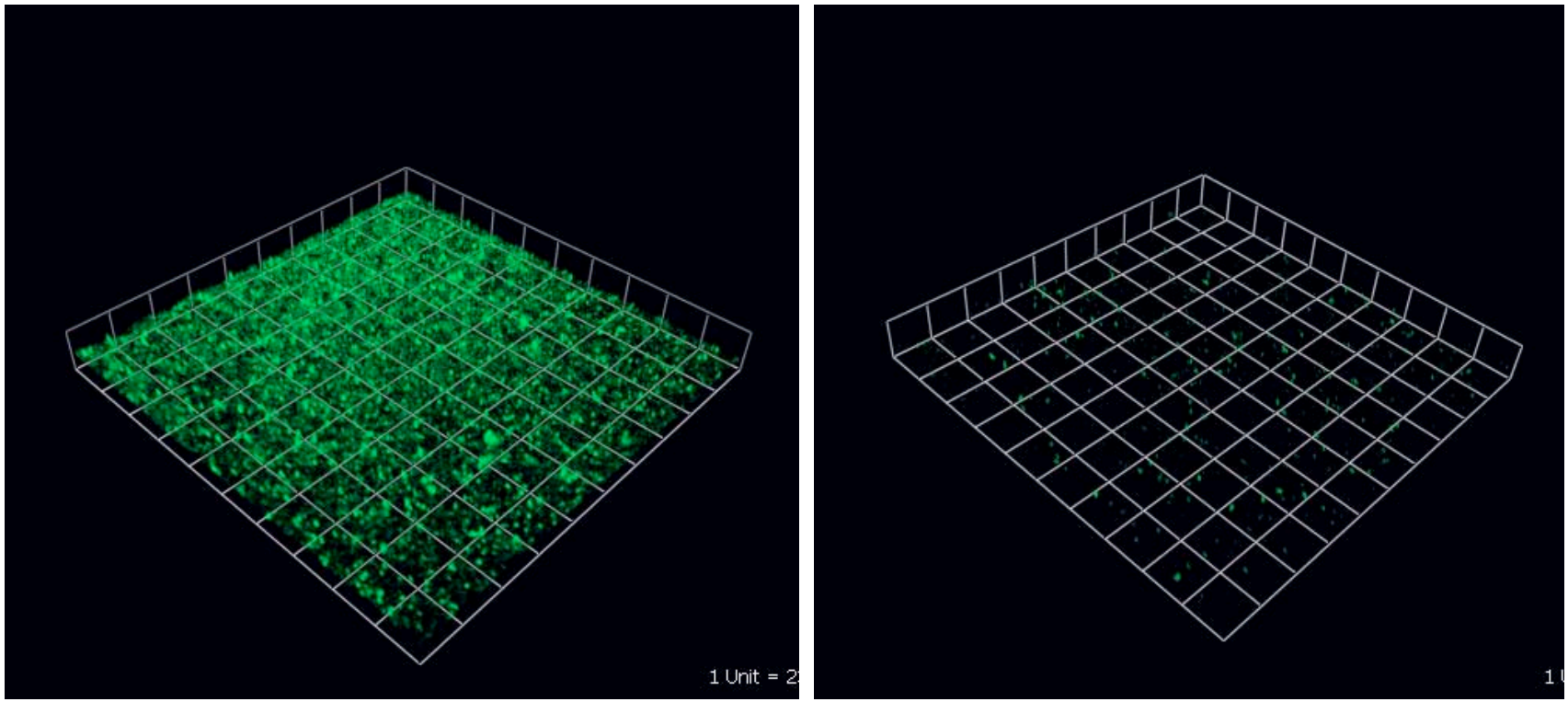
R. palustris biofilms produce hydrogen



R. palustris wild-type cells were grown with 4-hydroxycinnamate under nitrogen-fixing conditions. After biofilms were formed, the liquid in the tubes was discarded and the biofilms were rinsed with buffer. The tubes were filled with buffer, acetate was added as an electron donor and the biofims were exposed to light,. Data are average of three or more cultures and error bars are shown.

Fig. 3

R. palustris biofilms in flow cell chambers



4-hydroxycinnamate

succinate